

FURTHER STUDIES ON ALLOCENTROTUS FRAGILIS, A DEEP-SEA ECHINOID¹

ARTHUR C. GIESE

Department of Biological Sciences, Stanford University, Stanford, California

In a previous paper some data on the natural history and breeding of a deep-sea echinoid, *Allocentrotus fragilis*, were presented (Booolootian *et al.*, 1959). Further studies are reported here, primarily to define more clearly the breeding season of the species, as well as to get further information on its nutrition.

BREEDING SEASON

The breeding season in the previous study appeared to coincide with winter (December to March) but remained uncertain because storms on Monterey Bay during the critical period interfered with collecting at the time the boat was available. For the present study the urchins were collected from the same beds and by the same methods as previously, and the gonad index (the ratio of gonad volume to wet weight times 100) was used to estimate the breeding condition of the specimen as before. The breeding activity has now been followed for almost three years, and although the data for each year are incomplete, pieced together for the entire period in Figure 1, they give support to the notion that a single breeding season occurs in this species. Perhaps several periods of spawning and redevelopment of eggs occur in a given individual of this species but monthly sampling does not give information on this point. However, the appearance of germinal vesicles in spawned-out ovaries suggests just this. It proved impossible to keep specimens in healthy condition in the laboratory for more than about a month, although it was noticed that the red-spot "disease" was much less frequent in animals kept in aquaria in the dark (Araki, personal communication). It may prove possible to keep the animals in the laboratory for a longer time once the most favorable conditions are discovered.

More decisive evidence for an annual cycle is obtained from a study of teased pieces of the gonads and from attempts to fertilize the eggs. Such a study indicates that although the gonad index may be high and sperms may appear in September, October and November, the eggs are almost all in the germinal vesicle stage, each with a large nucleus. Such eggs do not mature after shedding and in no case are they fertilized on addition of active and presumably mature sperm. In December most of the females had ripe eggs and only occasional germinal vesicles were seen among them. The eggs fertilized and developed into normal plutei. The same was found to be true during February and March. Some of the females examined

¹ I am indebted to Messrs. George Araki, Peter Glynn and Joseph Balusteri for collecting the *Allocentrotus*; to Messrs. John Lawrence and James Stanley for help with some of the chemical determinations; and to Mr. Albert Towle for help with some of the respirometric determinations.

had few eggs but those eggs which remained in the ovaries fertilized and developed normally, suggesting that they were only a remnant, the bulk of the gametes having been released. During the period immediately following the breeding season, few females had eggs and germinal vesicles again became apparent in some. Thereafter, all the teased gonads examined microscopically appeared indeterminate as to sex. Apparently the tissue had entered a resting stage. The gonads remain indeter-

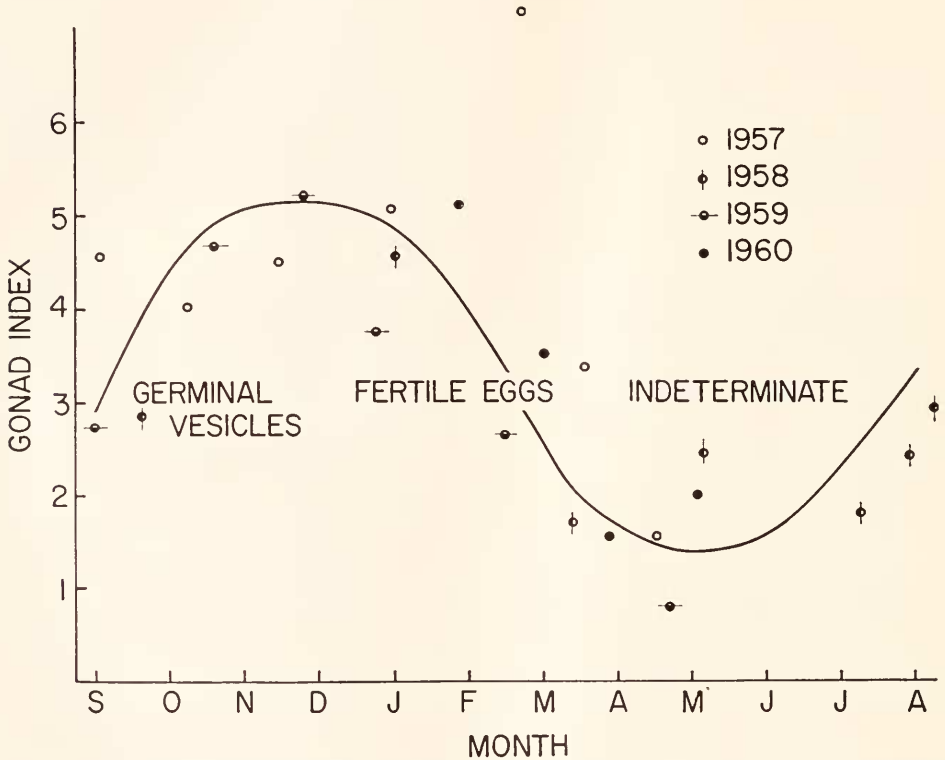


FIGURE 1. Reproductive cycle of *Allocentrotus fragilis* as measured by the gonad index (size of gonad relative to body weight) and presence and ripeness of the gametes. The gametes were studied closely only during 1959-60.

minate for several months but at the end of summer sperms can be seen and small germinal vesicles again make their appearance, long before the gametes form. Active sperms are present over a much wider span of time than mature eggs.

NUTRITION

Since the intestines of *Allocentrotus* brought in from the field are sometimes devoid of food, and at other times have very little, it would appear that the urchins may go for long periods of time without food. This seems likely since defecation may continue for a week or more in the laboratory, indicating slow digestion of an ample meal. In the previous study, on only one occasion were we fortunate enough

to obtain specimens richly charged with food when a diatom bloom occurred in the area. In the present study many collections yielded animals relatively full of diatoms, presumably because of similar blooms.

On November 7, 1960, a collection was made nearer the edge of the urchin bed in an attempt to get smaller specimens for a study of respiration. The intestines of these specimens were filled with bites out of large algae: green, red and brown. The fragments of algae were irregular and much larger than the balls of diatoms illustrated in the previous paper. In the foregut the algae were undigested and had little of the gelatinous material around them. In the hindgut more fully digested algae were enclosed in the mass of gelatinous material within which were

TABLE I

Chemical composition of gonads and gametes of Allocentrotus fragilis in per cent of dry weight

Tissue	Condition	Lipid	Non-protein N	Protein	Glycogen
Testis	gravid	14.54	3.26	28.70	0.36
		15.34	3.62	30.25	—
		13.08	4.24	31.93	—
Testis	starved animal	18.6	—	—	—
		17.7	—	—	—
Testis	spent	12.36	3.66	36.13	—
Sperm	—	3.60	4.22	38.7	—
Gonad	indeterminate	14.50	3.65	23.88	0.83
		17.85	3.66	27.05	—
Ovary	gravid	17.79	3.40	34.09	0.69
		14.61	4.40	32.47	—
		15.01	2.94	27.39	—
Ovary	starved animal	20.8	—	—	—
		20.0	—	—	—
Ovary	spent	12.83	3.17	20.87	—
Eggs	—	18.07	2.44	28.68	—

many bacteria, much as previously described in *Strongylocentrotus purpuratus* (Lasker and Giese, 1954). Fecal pellets collected from animals which had been in the laboratory aquaria overnight retained their shape and looked more like the rounded pellets previously described from *A. fragilis*. The algal fragments in many of them were almost completely digested, only colorless pieces remaining. In only one previous collection of *Allocentrotus* had individuals with pieces of larger algae (*Cladophora*) in the gut been obtained, all the others having diatoms and fragments of various minute materials present at the bottom of the sea. The November 7th collection followed rough seas which may have torn algae from the rich offshore beds nearby, making them available to the urchins.

When *Allocentrotus*, kept in the laboratory without food for a month, were

dissected, they were found to be free of intestinal contents. Since in some collections urchins were observed free of food, it is likely that in their natural environment they are occasionally unable to get food for a comparable period of time. A store of nutrients is therefore necessary to maintain the urchins between the sporadic droppings of material from the surface waters. The sea urchin has three major organs in which storage might occur: gonads, intestine and body wall. The latter (hereafter called the test) consists of the test proper, the epidermis, the tissues of the coelomic lining and the water vascular system attached to it. Biochemical analyses²

TABLE II
Chemical composition of intestine, intestinal contents, and shell of sea urchins in per cent of dry weight

Tissue	Condition	Lipid	Non-protein N	Protein
<i>Allocentrotus fragilis</i>				
Intestine	well-fed	28.88	2.17	35.38
		26.81	3.13	31.22
		23.24	2.77	38.84
Contents	starved	20.6	—	—
	fresh meal	8.28	1.66	22.83
	feces (rectal)	1.87	0.33	7.15
Test	well-fed	1.79	0.22	5.66
	starved	0.9	—	—
		0.8	—	—
Diatoms*	entire	8	—	28.1
<i>Strongylocentrotus purpuratus</i>				
Foregut	well-fed	12.28	3.84	39.33
Hindgut	well-fed	12.52	3.30	33.94
Contents	fresh meal	3.88	0.34	10.70
<i>Strongylocentrotus franciscanus</i>				
Gut	well-fed	22.2	—	—
		18.7	—	—

* From Pease, 1932; 63.2% carbohydrate present in diatoms.

indicated that the fragile urchin, like the purple sea urchin (Giese *et al.*, 1958), stores considerable lipid and a small amount of glycogen in its tissues, as seen in the data in Tables I and II. Protein, the main structural constituent of protoplasm, is also present in considerable quantity, as expected in any tissue. It would appear that the main reserve food is lipid, glycogen being a minor reserve. Since relatively little sugar appears in the body fluid, the latter finding is perhaps not surprising.

²The methods employed were like those described elsewhere (Giese *et al.*, 1958). Many of the measurements were done in triplicate; later only duplicates were run since the repeats were so much alike.

The data in Table I show that considerable lipid is present in the gonads of *Allocentrotus*, a bit more in the gravid than in the spent ones. There is more lipid in the eggs than in the ovary but relatively little in the sperms taken alone. Without comment, the data in Table I do not express fully the meaning of the changes in the organic content of the gonads of the animals during the breeding season. The gonad index varies by a factor of at least 5 (Fig. 1), the maximal variability being 9-fold. Therefore, the lipid content per unit dry weight of a gonad is not a true measure of the reserves, since the shrunken gonad of a spent or indeterminate gonad may be only one-fifth the size of the gravid gonad. The total lipid present in the gonad of a gravid animal would be at least 5 times as great as in a spent animal.

TABLE III
*Wet and dry weight of tissues and tests**

Tissue	Wet wt.	Dry wt.	% solid	% water	GI**	% body fluid
<i>Allocentrotus fragilis</i>						
Whole	35.6	3.8	10.6	89.4	2.06	58
Whole	66.9	6.25	9.4	90.6		
Whole	96.6	11.38	11.8	88.2	2.63	62
Gonad (♂)	2.54	0.51	20	80		
Gut	2.1	0.66	31.3	68.7		
Lantern	1.75	1.06	60	40		
Test	22.7	7.45	32.8	67.2		
<i>Strongylocentrotus purpuratus</i>						
Whole	98.3	32.55	33.2	66.8	8.6	22.3
Whole	64.6	24.5	38	62	10.83	30.3
Gonad (♀)	7	1.45	20.6	79.4		
Gut	2.64	0.95	36	64		
Lantern	2.2	1.6	73	27		
Test	3.2	20	61	39		

* Some data for *Strongylocentrotus* are given for comparison to *Allocentrotus*. Note the more massive skeleton in *Strongylocentrotus*, its lesser water content, and the lesser amount of body fluid.

** GI refers to gonad index (defined in the introduction).

Lipid is stored in quantity in the intestine and body wall. Analyses indicate that as much as 29% of the dry weight of the gut may consist of lipid, but only about 2% of dry weight of the test and its tissues consists of lipid.

While the per cent lipid content of the test appears small, it must be remembered that the test forms a considerable part of the entire dry weight of the urchin (Table III). An urchin which when wet weighs 96.6 grams, weighs only 11.38 grams when dry, including the body fluid salts, indicating that 88.2% of its wet weight is water. The dried gonads weigh 0.51 gram and the intestines, washed free of gut contents and dried, about 0.66 gram. The dry test and lantern weigh 9.5 grams. According to the data in Table II about 1/20 of the test (5.66%) is protein, which is probably an approximate measure of the amount of tissue present. Therefore,

about $5.66\% \times 9.5$ grams, or 0.54 gram of tissue, is probably present in the test. The amount of tissue in the test is thus probably equal to, or greater than that in the gut (no account was taken of the other organic constituents in the above calculations). It is possible that some protein forms a network in the test, in the interstices of which the salts are deposited, since the echinoderm test is supposedly a mesodermal structure like vertebrate bone (Hyman, 1955).

To compare the relative stores of lipids in gonad, test tissue, and gut, it is only necessary to multiply the amount of each tissue by its content of lipid. In this respect it appears that 9.5 grams \times 1.79% or 170 mg. are stored in the test and lantern, 0.5 grams \times 15.5% or 77 mg. are stored in the gonad, and 0.66 grams \times 26.8% or 176 mg. are stored in the gut of an animal which weighs 96.6 grams wet weight. This shows that in regard to its storage of lipid, the test and intestine may be of equal importance in an animal of intermediate gonad index (2.63) such as the one tested here. For an animal with an index of 5 the amount of lipid stored in the gonad would be increased by a factor of almost 2, making it about equal to the gut or test. For an animal of low index (1.5 to 0.8) the amount of lipid stored in the gonad would be reduced by a factor of 1/2 or 1/3, and the stores in the intestine and the tissues of the body wall would then be of even greater importance.

Since so much lipid is stored in the intestinal walls, it seems most likely that the food is either the source of the lipid or that the lipid is manufactured from carbohydrates or protein in the diet. The gut contents vary in lipid, a relatively fresh meal of diatoms containing 8.28% of lipid, whereas a well-digested mass of material contains only 1.87% of lipid (Table I). Pease (1932) lists the lipid content of diatoms as 8% of the dry weight.³ It therefore seems likely that the lipid probably is obtained from the diatoms and other food eaten by the urchin. The lipid is probably digested and stored, some of it in the gut, some in the other tissues.

In this respect it is interesting that another animal feeding upon diatoms, the sipunculid worm *Phascolosoma agassizi*, also has large stores of lipid in its gut, about 25% of the dry weight (A. Towle, personal communication). On the other hand, the data in Table II show that the purple sea urchin, *Strongylocentrotus purpuratus*, which feeds upon larger algae, stores only half as much lipid in its gut as *Alloccentrotus*. Nonetheless, lipid is prominent and a fairly large amount is present in the intestinal pellets, 3.88% of the dry weight in a fresh meal in the intestine. It is surprising in this regard that *S. franciscanus*, which has a diet much like *S. purpuratus*, has much more lipid stored in its intestine (Table II). Algae may at times also accumulate considerable lipid (Milner, 1953; Fogg and Collyer, 1954). Perhaps *S. franciscanus* has a diet richer in lipids than *S. purpuratus*.

How the nutrient gets from the gut to the other tissues is not known at present. Lipid may pass out as small droplets of fatty material like the chylomicrons of mammals or it might be carried out and distributed by wandering cells. Lipid is present in the body fluid but the exact amount has not been determined for lack of adequate methods.

³ Diatoms in culture do not always have this much lipid. According to Barker (1935) diatoms in a culture in the laboratory first synthesize carbohydrates during photosynthesis, the ratio of oxygen production to carbon dioxide consumption being unity. However, as the diatoms age they accumulate oil which is visible in droplets.

Upon starvation, stored nutrients are utilized. This was most evident in the shrinkage of the gonads of starved animals. Seven *Allocentrotus* starved for a month in an aquarium supplied with running sea water showed somewhat shrunken gonads—at least the index for the animals at the time of collection on November 6 was 3.2—a month later one would expect it to have risen to about 4.7; instead the gonad index for the starved animals was 2.5 on December 7. Similar shrinkage had previously been noted for the purple shore urchin (Lasker and Giese, 1954). It is probable that the nutrients stored in the gonads had been resorbed. While it seemed likely that the lipids were preferentially utilized, biochemical analysis revealed that per unit weight lipid increased in amount in the gonad, although it decreased both in total quantity in the animal and per unit weight in the body wall and the intestine. While the increase in lipid content per unit weight in the gonad may appear paradoxical, it is to be expected if one or more of the other nutrients in the gonad are utilized at a more rapid rate than the lipids. Since the total bulk of the gonad shrinks and the only other major organic material present in the gonad

TABLE IV
*Respiration of tissues of Allocentrotus**

Tissue	O_2 consumed μl./mg./hr.	R.Q.	Water content per cent	Number of experiments
Test	0.076	0.58	65.6	2
Testis	0.570	0.92	80.4	3
Ovary	0.088	0.65	80.8	8
Gut	0.383	0.57	79.2	10

* Note the high respiratory rate for the testis and gut as compared to the ovary and test.

is protein, proteins are probably being selectively metabolized in the gonads during the period of starvation. Wilber (1947) has described similar results after starvation of *Phascolosoma gouldii*.

RESPIRATION

A few studies were made of the respiration of *Allocentrotus* tissues, primarily with a view of determining how it compared with other marine animals. The respiratory quotient was determined to ascertain, if possible, what types of foods were being used by the urchins.

The data are given in Table IV. It is at once apparent that the rate of tissue respiration is comparable, per unit wet weight, to that for other sea animals (Nicol, 1960, p. 152). Of greater interest is the respiratory quotient characterizing the respiration of the sea urchin. If lipid is of importance in the economy of the sea urchin, a respiratory quotient of about 0.7 might be expected. If the urchin uses carbohydrates or mixtures of these with proteins and lipids, the respiratory quotient should be higher, approaching 1.0 when only carbohydrate is utilized.

Determination of the respiratory quotient of entire animals proves difficult because at the end of an experiment it is necessary to liberate the carbon dioxide which is trapped in the buffering system of the sea water bathing the urchins. Since to do this the sea urchins must be removed from the vessel, the extra manipulations

may permit the sea water to equilibrate with the air. Furthermore, the urchins shed some spines, pedicellariae or other skeletal pieces containing lime salts. Consequently, it is necessary to filter such water through bolting cloth to get rid of the calcareous materials. This involves still another step during which equilibration of the bathing water can occur with air, further vitiating the correction. The R.Q. for an entire animal small enough to fit into a Warburg flask was 0.7, suggesting lipid utilization. However, if any carbon dioxide had accumulated in the sea water during respiration, the manipulations preceding addition of acid and measurement might have liberated it, favoring a lower R.Q. value. Therefore, the data cannot be considered satisfactory since the method is unsatisfactory.

To by-pass this difficulty the gonad and gut tissues of the urchins were removed from animals, washed in sterile sea water, and the pressure changes measured manometrically in the presence of KOH in one series, and in the absence of KOH in another. In the latter case 3 N sulfuric acid was contained in the side arms, and at the end of the experiment the acid was added to liberate the excess carbon dioxide contained in the sea water surrounding the tissues. The data for respiration of the tissues in Table IV are therefore more satisfactory than those for the entire animal. Excepting the testes, the R.Q. for the tissues is between 0.6 and 0.7, definitely suggesting lipid metabolism. Since the experimental deficiencies mentioned for the studies on the entire animal do not apply, the data on tissues are more convincing than those for the entire animal. Presumably the respiration of the entire animal is the sum of the respiration of its various parts (Field *et al.*, 1939). Consequently, one might suppose that the data for the tissues are applicable to the entire sea urchin. The high R.Q. for the testes could result from utilization of carbohydrate along with some other nutrients.

DISCUSSION

The present study suggests that lipid may play a significant role in the economy of *Allocentrotus*. The sea urchin has a considerable supply of lipid in its usual diet of diatoms. It stores considerable lipid in its intestine and gonad and some even in the tissues adherent to the skeleton. Furthermore, the stores of lipids decrease in amount when the sea urchin is starved for a month. The amount of lipid is thus closely related to the nutritive state of the animal. This has proven to be the case in other echinoderms from this area (Giese, 1959) and in other regions as well (unpublished).

The small amount of glycogen found in tissues of *Allocentrotus* suggests that either some other kind of carbohydrate is stored in this urchin or else that carbohydrate plays a minor role here. Glucose does not increase the respiration of gut or gonadal tissue here, just as it failed to do in tissues of a purple sea urchin.

The respiratory quotient for the tissues studied—gut, test and ovary—is about 0.6 to 0.7. This indicates that some lipid is being used for respiration; that is, it is being metabolized. Addition of glucose does not change the R.Q. It thus appears possible that lipid is being used preferentially for metabolism although it is more likely that added sugar fails to stimulate respiration because it fails to enter the tissues.

In view of the apparent reliance of *Allocentrotus* on lipids, its occasional eating of large algae—red, brown and green—which have little lipid, but much polysac-

charide, is interesting. Either *Alloccentrotus* uses only the readily digestible materials in the algae or it has enzymes to utilize some polysaccharides, as does the purple sea urchin (Huang and Giese, 1958; Eppley and Lasker, 1959). The algae in the fecal pellets of *Alloccentrotus* feeding on large algae were rather completely digested, suggesting that more than the lipids are being utilized. It would be interesting to know whether the urchin itself has such enzymes, and secondly, whether the monosaccharides obtained are stored as polysaccharides in the urchin or are converted to lipid.

The observation that *Alloccentrotus* takes in large algae indicates that it is more resourceful than had been previously considered (Booolootian *et al.*, 1959). In the laboratory it failed to eat the large algae but apparently it does so when it gets them in nature.

While *Alloccentrotus* can withstand starvation at least a month, and probably longer, judging from the healthy appearance of the specimens starved (in the dark) for over a month, in nature this is probably not the rule since dissected animals usually had at least a few pellets in the gut. Specimens choked with pellets of algae at the time they were collected had nothing whatsoever in the gut.

No clear-cut correlation between the breeding season and the availability of food has been observed in the monthly collections. An annual reproductive cycle is suggested by the present study but the stimulus to the development of the gonads still remains elusive.

SUMMARY

1. Additional data on the size of the gonads relative to the body and the presence and ripeness of gametes and their maturity were gathered for a year on the deep-sea echinoid, *Alloccentrotus fragilis*.

2. The data indicate that the breeding season is an annual cycle with a maximum gonadal size in January and February, accompanied by the presence of mature eggs and sperm.

3. The sea urchins in one collection were found to have fed on large algae, the pellets resembling those of the intertidal sea urchin, *Strongylocentrotus purpuratus*.

4. A considerable quantity of lipid is found to be stored in the wall of the gut, less in the gonad, and still less in the body wall (per unit dry weight). Total amount of stored lipid is largest in the gut, next in the test, and least in the gonad.

5. The usual diatom diet of the sea urchin contains much fat; the algae tested in one series contain considerably less.

6. The respiratory quotient of the gut, ovary and test of the sea urchin was found to be about 0.6 to 0.7, suggesting utilization of lipids. The R.Q. for the testis was 0.92.

7. Some comparisons are made between *Alloccentrotus* and the purple intertidal urchin, *Strongylocentrotus purpuratus*.

LITERATURE CITED

- BARKER, H. A., 1935. Photosynthesis in diatoms. *Arch. Mikrobiol.*, 6: 141-156.
BOOLOOTIAN, R. A., A. C. GIESE, J. S. TUCKER AND A. FARMANFARMAIAN, 1959. A contribution to the biology of a deep-sea echinoid *Alloccentrotus fragilis* (Jackson). *Biol. Bull.*, 116: 362-372.

- EPPLEY, R. W., AND R. LASKER, 1959. Alginase in the sea urchin, *Strongylocentrotus purpuratus*. *Science*, **129**: 214-215.
- FIELD, J., II, H. S. BELDING AND A. W. MARTIN, 1939. An analysis of the relation between basal metabolism and summated tissue respiration in the rat. *J. Cell. Comp. Physiol.*, **14**: 143-157.
- FOGG, C. E., AND D. M. COLLYER, 1954. Accumulation of fats as a characteristic of certain classes of algae. *Congr. intern. bot. Paris, Rapports. et communs.*, **28**: 12530a. *Chem. Abstr.*, **48**: 11568.
- GIESE, A. C., 1959. Comparative physiology: annual reproductive cycles of marine invertebrates. *Ann. Rev. Physiol.*, **21**: 547-576.
- GIESE, A. C., L. GREENFIELD, H. HUANG, A. FARMANFARMAIAN, R. BOOLOOTIAN AND R. LASKER, 1958. Organic productivity in the reproductive cycle of the purple sea urchin. *Biol. Bull.*, **116**: 49-58.
- HUANG, H., AND A. C. GIESE, 1958. Tests for digestion of algal polysaccharides by some marine herbivores. *Science*, **127**: 475.
- HYMAN, L. H., 1955. The Invertebrates, Vol. 4. Echinodermata. McGraw Hill, New York.
- LASKER, R., AND A. C. GIESE, 1954. Nutrition of the sea urchin, *Strongylocentrotus purpuratus*. *Biol. Bull.*, **106**: 328-340.
- MILNER, H. W., 1953. Chemical composition of algae. *Carnegie Inst. Wash. Publ.* #60: 285-302.
- NICOL, J. A. C., 1960. The Biology of Marine Animals. Interscience Publ. Inc., New York.
- PEASE, H. D., 1932. The oyster: modern science comes to the support of an ancient food. *J. Chem. Ed.*, **9**: 1673-1712.
- WILBER, C., 1947. The effect of prolonged starvation on the lipids in *Phascolosoma gouldii*. *J. Cell. Comp. Physiol.*, **29**: 179-183.